Sodium Thiosulfate-Toxicity & Teratogenicity Studies in Avian Embryos-FDA Contract #72-345 No Date

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SODIUM THIOSULFATE

TOXICITY and TERATOGENICITY STUDIES in Avian Embryos

FDA Contract #72-345

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STUDIES on the TOXICITY and TERATOGENICITY of SODIUM THIOSULFATE in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Sodium thiosulfate (71-35) was found to be toxic to chicken embryos when administered in either the air cell or yolk. Employing air cell administration, dose levels of 110 mg/kg and above produced significant increases in embryo mortality when injected after 96 hrs incubation. In those eggs injected in the air cell prior to incubation, a level of 556 mg/kg was required to produce a significant increase in embryo mortality.

The injection of sodium thiosulfate into the air cell of 96 hr embryos significantly increased the incidence of abnormalities and those embryos showing head, limb, skeletal and visceral abnormalities. Teratogenesis of sodium thiosulfate appeared to particularly involve the head of the developing embryo.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Sodium thiosulfate (71-35) was solublized in deionized water for use in the four test protocols. The highest dose level employed was attained with a concentration of 556 mg/ml to produce a dose of 27.8 mg/egg (556 mg/kg).

MORTALITY

The mortality data for the four test protocols are shown in Tables 1 - 4. The highest dose level injected in the air cell (556 mg/kg) produced mortality rates of 29.91 and 98.41%, respectively, for the 0 and 96 hour times (Tables 1 and 2). Yolk injection at 0 hr produced a maximum of 52.17% mortality, while yolk-96 hr administration yielded 37.3% embryo deaths (Tables 3 and 4).

Chi-square analyses of the mortality data indicated that when administered in the air cell at 0 hrs sodium thiosulfate did not significantly ($P \geq 0.05$) increase mortality at a dose level of 220 mg/kg, while a dose of 556 mg/kg did significantly increase embryo deaths ($P \leq 0.05$). Air cell administration of sodium thiosulfate at 96 hrs caused a significant ($P < 0.05$) increase in embryo mortality at dose levels of 110 mg/kg and above (Table 5).

Sodium thiosulfate administered in the yolk at either of the injection times was toxic at a level of 556 mg/kg.

Among the four test protocols, a chi-square analyses of all doses in comparison with the solvent controls indicated a significant ($P < 0.05 - 0.005$) increase in chicken embryo mortality with sodium thiosulfate injections (Table 5).

Linear regression analyses of log dose against probit of mortality produced a significant linear relationship for only the two injection times (0 and 96 hrs) employed for air cell administration (Table 6). LD-50 estimates from the air cell series were 2467.8 mg/kg for the 0 hr injected groups and 93.2 mg/kg for the 96 hr groups.

Sodium thiosulfate was embryo toxic under the conditions employed in these studies.

TERATOLOGY

The occurrence of abnormal embryos and HLSV abnormalities among the four test protocols are shown in Tables 1 - 4. A total of 6.87% of the embryos injected with 110 mg/kg in the air cell at 96 hrs showed abnormalities. Chi-square analyses of these data indicated a statistically significant increase in the incidence of abnormalities at this dose level (Table 7). A comparison of all doses in this series with the solvent-injected control eggs also indicated a statistically significant ($P \leq 0.05$) increase in abnormalities with the injection of sodium thiosulfate (Table 7).

Probit analyses of these data resulted in a statistically significant linear relationship for only the air cell-0 hr series. These data yielded an estimate of 77503.4 mg/kg as the level which would produce a 50% incidence of abnormal embryos (Table 8).

Chi-square of HLSV abnormalities again yielded a significant relationship when all doses of sodium thiosulfate in this series were compared to the solvent control. Probit analyses of these data were not statistically significant. The individual teratological findings obtained in the four test protocols are shown in Table 10.

TABLE 1

SODIUM THIOSULFATE
in WATER
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
556.0	117	29.91	35	4.27	5	5.12	6	0.85	1			4.27	5								
220.0	119	21.00	25	1.68	2	0.84	1					0.84	1			0.84	1				
110.0	155	13.54	21	1.29	2	1.93	3	1.29	2			0.64	1								
22.0	119	16.80	20	0.84	1	0.00	0									0.84	1				
11.0	120	12.50	15	0.83	1	0.83	1					0.83	1								
0.0	156	17.30	27	1.28	2	1.92	3	1.28	2			0.64	1			0.64	1				
drilled	117	14.52	17	0.85	1	0.00	0									0.85	1				
untreated	285	12.63	36	0.70	2	1.75	5	0.70	2			0.70	2	0.35	1						

SUMMARY - ALL DOSE LEVELS

630	18.41	116	1.75	11	1.75	11	0.48	3			1.27	8			0.32	2				

TABLE 2

SODIUM THIOSULFATE
in WATER
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
556.0	126	98.41	124	0.79	1	0.79	1					0.79	1								
220.0	122	82.78	101	4.09	5	3.27	4	3.27	4									0.81	1		
110.0	160	61.87	99	6.87	11	6.25	10	6.25	10									1.25	2		
22.0	125	13.60	17	1.60	2	1.60	2	0.80	1			0.80	1								
11.0	126	14.28	18	0.79	1	0.00	0													0.79	1
0.0	164	9.75	16	0.60	1	0.60	1	0.60	1									0.60	1		
drilled	133	9.77	13	0.75	1	0.75	1	0.75	1							0.75	1				
untreated	285	12.63	36	0.70	2	1.75	5	0.70	2			0.70	2	0.35	1						

SUMMARY - ALL DOSE LEVELS

659	54.48	359	3.03	20	2.58	17	2.28	15		0.30	2			0.46	3	0.15	1

TABLE 3

SODIUM THIOSULFATE
in WATER
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
556.0	115	52.17	60	2.60	3	1.73	2	1.73	2											0.86	1
220.0	117	34.18	40	0.00	0	0.00	0														
110.0	119	44.53	53	0.84	1	1.68	2	0.84	1			0.84	1			0.84	1				
66.0	38	34.21	13	5.26	2	0.00	0											2.63	1		
22.0	117	30.76	36	2.56	3	1.70	2					1.70	2			0.85	1				
11.0	117	47.86	56	1.70	2	0.00	0									0.85	1			0.85	1
0.0	156	29.48	46	0.64	1	0.64	1	0.64	1												
pierced	60	8.33	5	1.66	1	0.00	0													1.66	1
untreated	285	12.63	36	0.70	2	1.75	5	0.70	2			0.70	2	0.35	1						

SUMMARY - ALL DOSE LEVELS

623	41.41 258	1.77 11	0.96 6	0.48 3		0.48 3		0.48 3	0.16 1	0.32 2	

TABLE 4

SODIUM THIOSULFATE
in WATER
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
556.0	126	37.30	47	0.79	1	0.00	0													0.79	1
220.0	125	24.00	30	3.20	4	2.40	3	0.80	1			0.80	1	0.80	1					0.80	1
110.0	160	29.37	47	1.87	3	1.87	3	1.87	3							0.62	1				
22.0	129	26.35	34	3.87	5	1.55	2	0.77	1			0.77	1			0.77	1	1.55	2		
11.0	128	20.31	26	3.12	4	1.56	2	0.78	1			0.78	1			0.78	1	0.78	1		
0.0	167	19.76	33	2.39	4	0.59	1					0.59	1			0.59	1	1.19	2		
pierced	114	16.66	19	0.87	1	0.87	1					0.87	1								
untreated	285	12.63	36	0.70	2	1.75	2	0.70	2			0.70	2	0.35	1						

SUMMARY - ALL DOSE LEVELS

668	27.54	184	2.54	17	1.50	10	0.90	6		0.45	3	0.15	1	0.45	3	0.45	3	0.30	2

TABLE 5

SODIUM THIOSULFATE
in WATER
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
11.0	0.87	1.01	8.88*	0.00
22.0	0.00	0.69	0.01	1.45
66.0	-	-	0.14	-
110.0	0.58	93.82*	6.00*	3.58
220.0	0.39	151.35*	0.48	0.53
556.0	5.36*	220.76*	13.37*	10.27*
All Doses (DF)	16.53*(5)	413.16*(5)	24.55*(6)	15.12*(5)

* Probability < 0.05 - 0.005.

TABLE 6

SODIUM THIOSULFATE
in WATER
PROBIT ANALYSES - MORTALITY

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
	mg/kg	mg/kg		
LD-30	1151.4	56.6	NS	NS
(Range)	(742.0 - 6810.2)	(35.9 - 75.4)		
LD-50	2467.8	93.2	NS	NS
(Range)	(1214.8 - 55702.1)	(68.7 - 116.7)		
LD-70	5289.3	153.4	NS	NS
(Range)	(1954.9 - 463524.1)	(123.0 - 193.3)		
LD-90	15902.8	314.9	NS	NS
(Range)	(3852.5 - 9965344.0)	(242.4 - 471.0)		

TABLE 7

SODIUM THIOSULFATE
in WATER
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
11.0	0.05	0.28	0.06	0.00
22.0	0.06	0.06	0.64	0.16
66.0	-	-	1.79	-
111.0	0.25	7.24*	0.27	0.00
220.0	0.06	2.62	0.02	0.00
556.0	1.35	0.28	0.67	0.35
All Doses(DF)	6.18(5)	19.28*(5)	8.22(6)	3.21(5)

* Probability $<$ 0.05 - 0.005.

TABLE 8

SODIUM THIOSULFATE
in WATER
PROBIT ANALYSES - ABNORMALITIES

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
	mg/kg			
LD-30	20468.5	NS	NS	NS
(Range)	(2908.5 - --)			
LD-50	77503.4	NS	NS	NS
(Range)	(5828.0 - --)			
LD-70	293465.3	NS	NS	NS
(Range)	(11634.1 - --)			
LD-90	2006738.0	NS	NS	NS
(Range)	(31472.0 - --)			

TABLE 9

SODIUM THIOSULFATE
in WATER
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
11.0	0.05	0.02	0.02	0.05
22.0	0.27	0.06	0.06	0.05
66.0	-	-	0.59	-
110.0	0.25	6.23*	0.27	0.30
220.0	0.06	1.56	0.02	0.64
556.0	1.35	0.28	0.07	0.02
All Doses (DF)	9.27(5)	19.10*(5)	4.92(6)	3.97(5)

* Probability $<$ 0.05 - 0.005.

TABLE 10

SODIUM THIOSULFATE in WATER
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Control	285	2	1	excencephaly; agenesis-wing, unilateral; cranio-rachischisis-abdomen
			1	anophthalmia-unilateral; dysgnathia-beak; celosomia-abdomen
Drilled Control - 0 hrs	117	1	1	dwarfism
Drilled Control - 96 hrs	133	1	1	anophthalmia-unilateral; dwarfism
Pierced Control - 0 hrs	60	1	1	cachexia
Pierced Control - 96 hrs	114	1	1	celosomia - abdomen
Air Cell - 0 hrs 56.0 mg/kg	117	5	1	celosomia-abdomen
			1	buphthalmia-unilateral; anophthalmia-unilateral; abnormal shortening-maxilla; dysgnathia-beak; fusion failure-abdomen
			3	fusion failure-abdomen
220.0	119	2	1	fusion failure-abdomen
			1	dwarfism
110.0	155	2	1	dysgnathia-beak
			1	anophthalmia-unilateral; celosomia-abdomen
22.0	119	1	1	agenesis-down
11.0	120	1	1	celosomia-abdomen
0.0	156	2	1	anophthalmia-bilateral; abnormal shortening-maxilla; dwarfism; celosomia-abdomen
			1	anophthalmia; exencephaly; abnormal shortening - maxilla

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TABLE 10
SODIUM THIOSULFATE in WATER
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 96 hrs	556.0 mg/kg	126	1	1	celosomia-abdomen
	220.0	122	5	1	microphthalmia-unilateral
				1	microphthalmia - bilateral
				1	buphthalmia-unilateral
				1	buphthalmia-bilateral
				1	edema
	110.0	160	11	3	buphthalmia-bilateral
				1	microphthalmia-unilateral
				1	buphthalmia-unilateral
				1	dysgnathia-beak; edema
				1	edema
				1	dysgnathia-beak
				1	buphthalmia-unilateral; exencephaly
				1	buphthalmia-unilateral; dysgnathia-beak
				1	microblepharia-eyelid, unilateral
	22.0	125	2	1	fusion failure-abdomen
				1	anophthalmia-bilateral; exencephaly; dysgnathia-beak

Sheet 3

TABLE 10
SODIUM THIOSULFATE in WATER
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 96 hrs	11.0 mg/kg	126	1	1	cachexia
	0.0	164	1	1	anophthalmia-unilateral; dysgnathia-beak; edema
Yolk - 0 hrs	556.0	115	3	1	anophthalmia-bilateral
				1	cachexia
				1	dysplasia-eyelid
	220.0	117	0	0	
	110.0	119	1	1	brachynathia-mandible; dwarfism; celosomia-abdomen
	66.0	38	2	1	edema
				1	necrosis-liver
	22.0	117	3	1	fusion failure-abdomen
				1	dwarfism
				1	celosomia-abdomen
	11.0	117	2	1	cachexia
				1	agenesis-down
	0.0	156	1	1	microphthalmia-unilateral
Yolk - 96 hrs	556.0	126	1	1	cachexia
	220.0	125	4	1	septation-ankle, bilateral
				1	fusion failure - abdomen
				1	exencephaly
				1	cachexia

Sheet 4

TABLE 10

SODIUM THIOSULFATE in WATER

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 96 hrs	110.0 mg/kg	160	3	1	fusion failure-skull
				1	microphthalmia-unilateral; dysgnathia-beak
				1	anophthalmia-bilateral; dwarfism
	22.0	129	5	1	umbilical cord around fetus
				1	edema
				1	dwarfism
				1	fusion failure-abdomen
				1	exencephaly
	11.0	128	4	1	hypopigmentation-down
				1	fusion failure -abdomen
				1	dwarfism
				1	anophthalmia-bilateral; abnormal shortening-maxilla
	1.0	167	4	1	agenesis-down
				1	fusion failure-abdomen
				1	edema
				1	umbilical cord around fetus